CONCERNING BRACHET'S IDEAS OF THE RÔLE OF MEMBRANE FORMATION IN FERTILIZATION.[1]

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1. A recent publication by Brachet[2] seems to make it necessary to discuss once more the relation between membrane formation and development. The writer had shown in 1895 that if oxygen is completely withdrawn from the fertilized sea urchin egg no development is possible, while the moment oxygen is admitted the development can begin again. As he had suggested in 1906 and as has since been proved by O. Warburg, and H. Wasteneys and the writer, the entrance of the spermatozoön into the egg of the sea urchin increases the rate of oxidations in the latter (by 400 or 600 per cent). The entrance of the spermatozoön causes also a membrane formation which is very marked in the fresh egg and is generally less marked or may appear to be absent if the egg has been lying in sea water for a day or more. It has been shown, moreover, that the artificial production of a membrane in the unfertilized egg by butyric acid has the same influence upon the increase of the rate of oxidations as the entrance of the spermatozoön. These and other facts seemed to support the view of the writer that an alteration of the surface of the egg, which usually but not necessarily results in a membrane formation, is an essential feature of the development of the egg.

More recent experiments by Warburg[3] have made it very probable that the process of oxidations in the sea urchin egg is a case of catalysis by iron, which is confined mainly if not exclusively to the surface; and this fact, in connection with the data mentioned above, seems to indicate that the process which underlies membrane formation in the unfertilized egg may consist in bringing about or rendering possible the iron catalysis which is

[1] From the Rockefeller Institute for Medical Research, New York.

[2] *Compt. rend. l'Acad. d. sc.*, CLIX., 642, 1914.

[3] Warburg, *Zeitschr. f. physiol. Chem.*, XCII., 231, 1914.

responsible for the sudden increase in the rate of oxidations after artificial or natural membrane formation. Since fertilization by sperm is accompanied by a membrane formation and followed by the same increase in the rate of oxidations as is artificial membrane formation by butyric acid, it is probable that the alteration of the surface (underlying membrane formation) is also the cause for the increase in the rate of oxidations in the process of natural fertilization.

2. The writer has time and again stated that the formation or non-formation of the fertilization membrane is of only secondary importance; what matters are the physicochemical changes which underlie the membrane formation and which are responsible for the sudden rise in the rate of oxidations of the sea urchin egg after artificial or natural membrane formation; and which may even occur when for some reason the fertilization membrane is modified or when its formation is entirely suppressed. There is no doubt that in the writer's first experiments with the purely osmotic method, the fertilization membrane was often very indistinct or in some cases even completely lacking, while nevertheless the enormous increase in the rate of oxidations and development to the pluteus stage ensued.[1]

It is possible to modify the surface of the unfertilized egg in such a way that if it is later fertilized by sperm the abnormal character of the membrane formed, or the abnormal conditions of the surface, may lead to the death of the egg. The writer described such a case in 1909.[2] When the unfertilized eggs of *Strongylocentrotus* were treated for five minutes with a hyperalkaline solution of NaCl (50 c.c. *m*/2 NaCl + 1.0 c.c. N/10 NaOH) and then transferred to normal sea water to which sperm was added, the eggs were all fertilized but apparently without membrane formation, though in reality probably with a tightly fitting membrane. They all segmented but perished in the blastula or gastrula stage. When, however, the eggs were not fertilized immediately after the treatment with alkali but after

[1] The literature of the subject can be found in the writer's recent book on "Artificial Parthenogenesis and Fertilization," Chicago, 1913.

[2] Loeb, "Die chemische Entwicklungserregung des tierischen Eies," Berlin, 1909, p. 117.

they had been in the sea water for one hour or more, a more normal membrane was formed and the eggs developed into plutei. Why did the eggs only live to the blastula or gastrula stage when they were fertilized immediately after the alkali treatment? Should this have had something to do with the abnormal character of the membrane which was formed when the egg was fertilized immediately after the alkali treatment? Were the cells pressed by the membrane which was too tight, and did this pressure kill them if prolonged? If this were the case, a tearing of the membrane should save the life of the egg. It would be of interest to try this experiment.

3. In a recent number of the *Comptes rendus de l'Académie des Sciences*, Brachet has published an observation which may or may not be similar to the one just mentioned. He found a year ago that if the eggs of the sea urchin, *Paracentrotus lividus*, at Roscoff, are put for two hours in contact with sperm of *Sabellaria alveolata* (which cannot fertilize the eggs) and if they are afterwards fertilized with sperm of their own species, they develop without apparently forming a fertilization membrane. From this Brachet concludes that the formation of a fertilization membrane is not necessary for development, a conclusion which will surprise nobody who is familiar with my first experiments on artificial parthenogenesis, or who has ever fertilized eggs which have been lying in sea water for several days. Moreover, Brachet observed that the sea urchin eggs which are fertilized with sperm of their own species, after two hours' treatment with the sperm of *Sabellaria*, die at the time of gastrulation. The prolonged treatment of the eggs of *Paracentrotus* with the sperm of *Sabellaria* seems therefore to have a similar effect as the short treatment of the egg of *Strongylocentrotus* with the alkaline NaCl solution in my experiments.

4. The deductions which Brachet draws seem, however, difficult to reconcile with each other. We stated already that he assumes that the eggs of *Paracentrotus* after two hours' treatment with the sperm of *Sabellaria* form no fertilization membrane after fertilization with their own sperm. Yet, he states further that these eggs die in the gastrula stage for the reason that they cannot hatch; for if he shakes the eggs and thereby destroys "la couche

corticale" the larvæ can hatch and are now able to develop into plutei. The only membrane, however, which can prevent the eggs from hatching is the fertilization membrane, and it is impossible to harmonize the two statements of Brachet, first, that these eggs have no fertilization membrane and, second, that the gastrulae cannot hatch unless the membrane of the egg is pierced. Professor Goldschmidt, to whom I showed Brachet's paper suggested that Brachet probably means by "couche corticale" the hyaline membrane (Herbst's "Verbindungsmembran") which surrounds the blastomeres and that he assumes erroneously that this hyaline membrane forms a continuous layer around the blastula in the same way as the fertilization membrane does. This is, however, not the case since the hyaline membrane participates in the process of segmentation and forms a distinct layer around each individual blastomere, but not a continuous envelope around the whole blastula.

Brachet's observation is intelligible on the assumption that the egg after it has been treated with the sperm of *Sabellaria* forms a very tightly fitting membrane when it is fertilized with its own sperm and that this membrane must be torn by shaking the egg in order to allow the blastula to hatch (or to escape from being killed by the mechanical pressure of the tightly fitting membrane?). Brachet found also that it is possible to substitute for the shaking of the egg a treatment with butyric acid, which as he assumes also tends to remove the obstacle to the hatching. This may be correct, but unfortunately he draws the further conclusion that the butyric acid treatment must have the same effect upon the unfertilized egg as upon the fertilized egg which has previously been treated with the sperm of *Sabellaria*. Leaving aside the fact that the unfertilized egg has no membrane, it has been shown that the butyric acid treatment raises the rate of oxidations of the *unfertilized* egg about 400 or 600 per cent., while acid does not increase, but, on the contrary, lowers the rate of oxidations in the *fertilized* egg. Moreover, the writer has shown that if a *fertilized* egg is treated with butyric acid, in the same way as is required for inducing artificial parthenogenesis, the *fertilized* egg is not injured, while the inducing of a membrane formation by butyric acid in the *unfertilized* egg leads

to the rapid death of the latter, if it is kept at room temperature and if it does not receive a second treatment either with a hypertonic solution or lack of oxygen. This case was fully discussed by the writer in a recent paper.[1] It is therefore not justifiable to conclude that the action of butyric acid on the unfertilized egg must be identical with the action of the same substance on a fertilized egg, treated beforehand with the sperm of *Sabellaria*.

Should it be possible that Brachet's "couche corticale" is the chorion or the "jelly" which surrounds the unfertilized egg? But this jelly is normally dissolved when the egg is fertilized. It might be conceivable that the sperm of *Sabellaria* causes a hardening and a contraction of this jelly which protects it against being dissolved by the sperm of the sea urchin and that subsequent shaking or a subsequent treatment with acid destroys this jelly. But granted this were the case, it would be erroneous to use experiments on an artificially altered chorion to draw conclusions upon the rôle of membrane formation in fertilization or artificial parthenogenesis.

The writer wonders how Brachet (or Herlant) are going to harmonize the following well-established facts with their views. If the eggs of *Strongylocentrotus purpuratus* are treated with hypertonic sea water for about two hours, they form in most cases no membrane and nothing happens to them except that a certain percentage of them begin to divide very regularly into 2, 4, 8, possibly 12 or 16 cells and then stop. Such eggs are to all appearances in the resting stage and live as long as the other unfertilized eggs if nothing is done to them. If they are fertilized by sperm each blastomere forms a special fertilization membrane and now each blastomere develops into a blastula or into a pluteus, according to the size of the blastomere. They also develop into plutei if an artificial membrane formation is called forth with the aid of butyric acid. The writer is inclined to explain this phenomenon by assuming that the treatment with the hypertonic solution called forth two effects, one of which was a peripheral change resulting in an increase in the rate of oxidations. This effect is, as the writer has shown, reversible

[1] Loeb, "Weitere Beiträge zur Theorie der künstlichen Parthenogenese," *Arch. f. Entwcklngsmech.*, XXXVIII., 409, 1914.

and was possibly reversed while the eggs were in an early stage of development. It seems to the writer impossible to reconcile these observations with the purely morphological views of Brachet or Herlant.

Brachet (like Herlant) tries to explain the phenomena of artificial parthenogenesis and fertilization without any consideration of the striking chemical processes that accompany fertilization and artificial membrane formation. He reverts to that standpoint of the pure morphologist which Sachs, in his papers on "Matter and Form in Plants" characterized as "empty formalism." This standpoint disregards the sources of energy in life phenomena and treats morphological changes as if they required no source of energy. It seems to the writer that the fact of the necessity of oxygen for development, the fact that mere membrane formation (both by butyric acid or by a spermatozoön) raises the rate of oxidations 400 or 600 per cent, and the fact that the amount of rise is identical in both cases, are so striking, that these facts cannot be ignored in a theory of the rôle of membrane formation in the development of the sea urchin egg. The writer has always considered the changes underlying the membrane formation as the essential factor in the initiation of development, while he considered the formation of a fertilization membrane only as a welcome but not essential indicator of the chemical changes in the surface of the egg; a fact which Brachet, on account of his disregard for the chemical processes, has entirely overlooked. Brachet, from his purely morphological standpoint, erroneously assumes or makes it appear as if I considered the formation of a visible membrane as the only and essential act in the initiation of development.